ULTRAVIOLET LIGHT AND THE DEVELOPMENT OF FUCUS EGGS AS AFFECTED BY AUXIN AND pH [1]

D. M. WHITAKER

(From the Department of Biology, Stanford University)

INTRODUCTION

It has been shown earlier (Whitaker, 1941) that unilateral irradiation with suitable doses of a number of wave-lengths of ultraviolet light (2345, 2482, 2537, 2654, 2804, 3130, 3660 Å) causes *Fucus* eggs to develop rhizoids on their non-irradiated halves. In the case of the wave-length 2804 Å, it was further shown that the responsiveness of the eggs increases gradually after fertilization, reaching a maximum in about 7 hours, and that the response of a population of eggs is proportional, over a wide range, to the logarithm of the dosage. Sufficiently strong doses inhibit rhizoid formation altogether. In an earlier paper in which these results were reported (Whitaker, 1941), it was suggested that inactivation or destruction of growth substance (auxin) in the eggs might be the principal means of action of the shorter wave-lengths of ultraviolet. This would suppose that moderate doses determine polarity by differentially destroying auxin on one side of the egg, and that strong doses might prevent rhizoid formation by destroying auxin throughout the egg. If this were so, it might be expected that adding auxin (β-indole acetic acid) to the medium after irradiation would tend to counteract the effects of ultraviolet.[2] The present experiments make this test. Experiments are carried out in sea water acidified to pH 6.0 (which approaches the limit of acid tolerance of the *Fucus* egg) as well as in normal sea water at pH 8.0 because the penetration of β-indole acetic acid into the eggs is favored by reduced dissociation in acid medium. Auxin is also more effective physiologically under acid conditions, i.e. in the molecular form (Bonner, 1934).

The effect of pH of the medium on the response of the eggs to ultraviolet was also determined to provide further information about the reactions in the protoplasm caused by the ultraviolet.

[2] This might be expected even if a uniform application of auxin did not abolish a gradient of concentration in an egg irradiated with a moderate dose from one side, if enough auxin is supplied so that the concentration at the irradiated surface reaches or passes an optimum.

The shorter wave-lengths of ultraviolet are heavily absorbed by the protoplasm of the *Fucus* egg (Whitaker, 1941) so that the immediate effect of the ultraviolet must be greatest in the cortical region on the irradiated side of the egg. This raises a question that is broader than the rôle of auxin. Do the rhizoids form on the non-irradiated halves of the eggs because the ultraviolet so damages the cortex upon which it falls that rhizoid formation becomes impossible? If so, the rhizoids would have to form, if at all, on the non-irradiated halves of the eggs, as observed. One series of experiments is designed to answer this question.

Material and Method

Fucus furcatus was collected at Moss Beach and at Pescadero Point, California, from December 1940 to July 1941, inclusive. The gametes were obtained by methods already described, and fertilization was caused to take place at a determined time, ± 7 minutes. Filtered sea water (Sp. gr. 1.026–1.027) at pH 7.9–8.1 was used as the medium throughout, except where otherwise specifically noted. When sea water was acidified to pH 6.0, approximately one-tenth part of Sorensen's buffer was added and the mixture was aerated with a scintered glass nozzle until pH equilibrium became established. No consequential change in specific gravity resulted. The eggs were reared in a constant temperature room at $15 \pm \frac{1}{4}°$ C., and were shielded from white light until the final results were observed.

The ultraviolet source was a gas tube mercury resonance lamp made by Westinghouse, and sold under the trade name Sterilamp. The lamp has a 10-inch tube made of Corex glass, which stops almost all of the λ 1800 Å band. The lamp produces practically no heat and more than 90 per cent of the radiant energy is of the wave-length 2537 Å. There are small amounts of λ 3130 and 3660 Å, and some bluish visible light. It has been shown earlier (Whitaker, 1941) that pure λ 2537 Å directs the position of rhizold formation in *Fucus*, and also that λ 3130 and 3660 are relatively ineffectual compared with the shorter wave-lengths of ultraviolet. For present purposes, therefore, the effects of the radiation from the Sterilamp can be considered quite dominated by the effects of λ 2537 Å.

Four clear fused quartz culture vessels of a considerable degree of optical perfection were made by the Hanovia Chemical and Manufacturing Company. These vessels measured $50 \times 25 \times 15$ mm., and eggs were irradiated and reared in single or double rows on slabs of plate glass resting on the bottoms inside the quartz vessels. When there were two parallel rows of eggs, the second row was placed on a glass shelf

above and behind the first row so that no eggs were eclipsed. In all cases the eggs within the rows were at least 5 egg diameters apart, and the ultraviolet light passed through $0.9 \pm .1$ cm. of medium before reaching the eggs. The culture vessels were maintained on a leveled glass platform so that the eggs did not roll, and a microscope was arranged so that the eggs could be observed at any time with red light without touching the platform or vessels.

The unit of dosage adopted in this work represents the application to the eggs of 1 erg per square millimeter, without correction for scatter and absorption in passing through the vessel wall and 9 mm. of sea water. The scatter and absorption are minor, and one unit very nearly represents the application of 1 erg per square millimeter to the surfaces of the eggs. The intensity of the ultraviolet shorter than λ 3200 Å emitted by the Sterilamp was measured by means of a Hanovia Ultraviolet meter. The intensity of the lamp source under working conditions did not vary more than about 6 per cent. Its intensity was measured from time to time during an exposure, and the variation was compensated for by adjusting the duration of exposure to give the desired total dose.

The distance of the Sterilamp, and the length of lamp tube used, were varied to obtain great range of effective intensity. When the dosage was 1–100 units, the lamp was 4 feet from the eggs; 4 cm. of lamp tube was used and energy was supplied to the eggs at the rate of about 1 unit every 16 seconds. When the dosage was 20,000–50,000 units, the lamp was 6 inches from the eggs, and energy was supplied to the eggs at the rate of about 525 units per minute. Individual shutters were used to interrupt the ultraviolet to each vessel, and when the vessels were relatively near the lamp, parallel side baffles ran from either end of each vessel to the lamp tube so that the eggs did not receive ultraviolet from too great an angular span of directions. The beam of ultraviolet approached the vessels with a downward slant of about 3° to insure against eclipsing by the forward margin of the slab upon which the eggs rested.

When strong dosages (20,000–50,000 units) of ultraviolet were applied, development was greatly delayed. Therefore, after such strong irradiations the eggs were transferred on the glass slabs to glass vessels for rearing, in order to free the quartz vessels for irradiating eggs for new experiments. There may have been some tendency for the eggs to roll during transfer, and partly for this reason the direction of rhizoid formation was not counted in the experiments with strong dosages. The retardation and inhibition of development was measured in these cases.

RESULTS

The Relation between Dosage and Response

Experiments were designed to test the relation between dosage of λ 2537 Å and the response of populations of eggs, in the lower dosage range in which the position of rhizoid formation is determined but development is not impeded. The eggs had been fertilized 8 hours when the irradiation began.

The sensitivity of different samples of eggs varies somewhat, especially as the season progresses. It was therefore necessary to cover nearly the whole span of dosages in each experiment, so that balanced averages might be obtained when all experiments were brought together. Four culture vessels in each experiment were exposed to different dosages, and the average results of 11 experiments are shown graphically in Fig. 1. The points at 1, 2, 5, and 10 units are each based on 550–670 eggs, and the point at 50 units is based on 340 eggs. It is clear that the response of populations is proportional, over a wide range, to the logarithm of the dosage.

This curve (Fig. 1) gives a basis for selecting the dosage for tests of the effect of auxin and pH on the sensitivity of the eggs to ultraviolet. Since 50 per cent of the rhizoids in a population form away from any given side of the culture vessel under conditions of randomness, it is the excess above 50 per cent forming on the halves of the eggs away from the source of radiation that measures the effect of the radiation. Seventy-five per cent represents half of the maximal response to ultraviolet, and while the dosage required to cause this response will vary somewhat, interpolation on Fig. 1 indicates that 2.8 units is a good dosage to use.

Rhizoid Formation on the Surface Irradiated with Ultraviolet

The question was raised at the end of the Introduction whether the irradiated surface of the egg is so damaged by destructive wave-lengths of ultraviolet that rhizoid formation is impossible on this surface. If so, in determining the developmental axis the ultraviolet might merely be acting through general breakdown of a non-specific nature. To find out whether the irradiated surface is capable of rhizoid formation, eggs were first irradiated from one side with 50 and 100 units of λ 2537 Å, since these dosages are more than adequate to cause rhizoids to form on the non-irradiated sides of the eggs (see Fig. 1). The midpoint of the period of irradiation was at 8 hours after fertilization, which is still many hours before rhizoids form (Whitaker and Lowrance, 1936). Immediately after the irradiation with ultraviolet, an ordinary 75-watt

bulb at 1 meter distance was turned on so that the eggs in half of the vessels were irradiated with white light from the opposite side. The white light was left on continuously until results were observed at 26 hours after fertilization. The response of *Fucus* eggs to unilateral irradiation with white light is to form the rhizoids on the sides of the eggs away from the light (Whitaker and Lowrance, 1936). The directional demands of the ultraviolet and of the white light were therefore
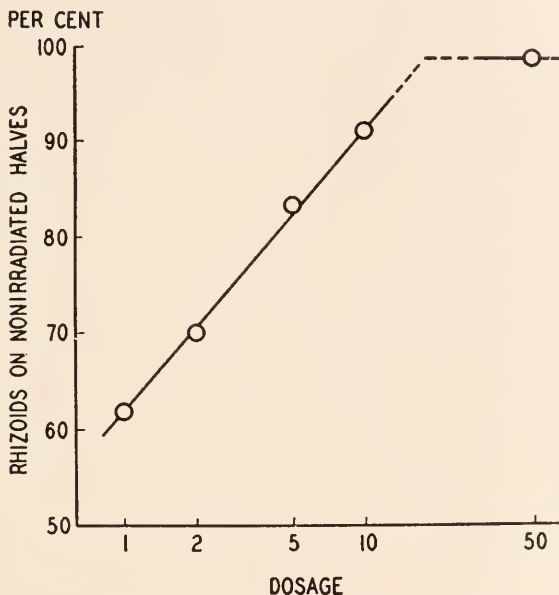
PER CENT

FIG. 1. The vertical axis indicates the percentage of eggs in populations that formed rhizoids on the halves away from the source of radiation after unilateral irradiation with λ 2537 Å. The horizontal axis shows the dosage on a logarithmic scale. One unit of dosage represents approximately 1 erg per square millimeter (see text).

opposed in these experiments, but the white light was left on much longer to dominate.

The results are shown in Table I. It is clear that the white light reverses the direction of rhizoid formation so that rhizoids form on the surfaces of the eggs that were irradiated with ultraviolet. This appears to indicate that the unilateral ultraviolet does not determine the position of rhizoid formation merely by causing extreme destruction and incapacitation of the irradiated surface of the egg. Some more specific effect is to be sought.

The Effect of Auxin on the Response to Low Dosages of Ultraviolet

A series of experiments was designed to test the effect of β-indole acetic acid (auxin) on the sensitivity of eggs to low dosages of λ 2537 Å that determine the polarity of some of the eggs in a population. Eggs were irradiated from one side with 2.8 units at 8 hours after fertilization. In each of 7 experiments two control vessels contained normal sea water throughout, while in two other vessels the medium was changed im-

Table I

Results of experiments in which eggs were irradiated from one side with 50 and 100 ergs per square millimeter of ultraviolet light (λ2537 Å). In the second part of each experiment, the irradiation with ultraviolet was followed by a more prolonged irradiation with white light from the opposite sides of the eggs.

Exp.	Total No. Eggs	Treatment	Rhizoids on Halves of Eggs Exposed to Ultraviolet
			per cent
1	238	50 ergs per sq. mm. unilateral ultraviolet	6
	220	50 ergs per sq. mm. unilateral ultraviolet followed by opposed white light	100
2	205	50 ergs per sq. mm. unilateral ultraviolet	2
	192	50 ergs per sq. mm. unilateral ultraviolet followed by opposed white light	100
3	466	100 ergs per sq. mm. unilateral ultraviolet	4
	352	100 ergs per sq. mm. unilateral ultraviolet followed by opposed white light	100
4	209	100 ergs per sq. mm. unilateral ultraviolet	0.5
	176	100 ergs per sq. mm. unilateral ultraviolet followed by opposed white light	100

mediately after irradiation to sea water containing 150 mg. per liter of β-indole acetic acid at pH 8.0. A small amount of NaOH was added to maintain pH at 8.0. This concentration of β-indole acetic acid was the greatest that could be used at pH 8.0 without considerably delaying the development of the eggs. In most but not all of the experiments the eggs in the solution containing β-indole acetic acid gave a somewhat greater response. The overall averages for the 7 experiments, involving 1230 control eggs in sea water and 1200 eggs in the β-indole acetic acid-sea water solution, show that 64 per cent formed rhizoids on the halves

away from the ultraviolet in normal sea water, while 69 per cent did so in the sea water containing β-indole acetic acid. The difference is significant. It can be concluded, however, that the effect of adding β-indole acetic acid, even in amounts beginning to be toxic, is slight, and it does not tend to decrease the effect of the ultraviolet.

Another series of experiments was carried out in which the same concentration of β-indole acetic acid was added some time before irradiation. Its presence greatly decreased the response of the eggs, but absorption measurements showed that this could be explained by the very great extinction of the ultraviolet in passing through 9 mm. of the solution before reaching the eggs.

Since β-indole acetic acid may not enter the eggs readily in its highly dissociated state at pH 8.0 (although it was physiologically active in retarding development), its effect has also been tested at pH 6.0, again using the strongest concentration that did not considerably delay development. The permissible concentration at pH 6.0 was much less: 5 mg. per liter. In both experimental and control vessels the eggs were placed in acidified sea water at $2\frac{1}{2}$ hours after fertilization. They were irradiated with 2.8 units of λ 2537 Å at 8 hours after fertilization, and immediately after the irradiation the β-indole acetic acid was added to half of the vessels. The averaged results of 4 experiments, involving 742 control eggs and 670 experimental eggs to which β-indole acetic acid was added, show that 70.6 per cent of the control eggs formed rhizoids on the halves away from the radiation, and 69.1 per cent of the experimental eggs did so. The results of the individual experiments agree with this average result, and it may be concluded that β-indole acetic acid does not increase the sensitivity of the eggs at all at pH 6.0.

Other experiments were carried out in which the β-indole acetic acid was added some hours before irradiation. These experiments gave similar results, although the presence of even 5 mg. per liter of β-indole acetic acid at the time of irradiation slightly reduced the dosage of ultraviolet received by the experimental eggs, by absorption.

The Effect of Auxin on the Inhibiting Action of Strong Dosages of Ultraviolet

The preceding series of experiments tested the effect of β-indole acetic acid on the sensitivity of the eggs to relatively small unilateral doses of λ 2537 Å, as indicated by the positions at which the rhizoids formed. The object of this series of experiments is to find out whether the effects of heavy doses of λ 2537 Å, which are so strong as greatly to delay and inhibit rhizoid formation and cell division, are mitigated or relieved by

the addition of β-indole acetic acid. If the delay and inhibition of rhizoid formation is due primarily to the destruction of auxin in the egg cells, addition of β-indole acetic acid after irradiation should relieve the symptoms. Four experiments were carried out in which the eggs received a dosage of 20,000 units. The irradiation began 7.7 hours after fertilization and lasted approximately 38 minutes. Immediately after the irradiation, 150 mg. per liter β-indole acetic acid was added to the medium of half the vessels. The pH of all media was 8.1.

Normal non-irradiated eggs have all formed rhizoids at 24 hours after fertilization (Whitaker, 1936). The eggs irradiated with 20,000 units were retarded, however, so that less than half had formed rhizoids at 48 hours when counts were made in the present instance. The averaged results of the four experiments, involving 790 control eggs and 780 experimental eggs to which β-indole acetic acid was added, show that 35.2 per cent of the control eggs had formed rhizoids at 48 hours after fertilization, and 36 per cent of the experimental eggs had done so. The difference is entirely without significance and the conclusion is that β-indole acetic acid does not revive heavily irradiated eggs, or relieve their retardation or inhibition. This conclusion is further supported by the results of a few experiments in which 40,000 and 50,000 units of λ 2537 Å were applied. These heavy dosages greatly retarded and inhibited rhizoid formation, but the addition of β-indole acetic acid (150 mg. per liter at pH 8.0; 5 mg. per liter at pH 6.0) had no effect.

The Effect of pH on the Response to Ultraviolet

The effect of hydrogen ion concentration at the cell surface upon the response to ultraviolet is of interest because it provides further information about the reactions brought about in the protoplasm by the ultraviolet. Therefore experiments were designed to compare the sensitivity of the eggs to λ 2537 Å when the pH of the medium was 8.0 and 6.0. In each of 7 experiments two culture vessels contained eggs in medium at pH 8.0 and two contained eggs in medium at pH 6.0. Eggs were placed in medium at pH 6.0 2½ hours after fertilization, and all eggs were irradiated with 2.8 units at 8 hours after fertilization. In every one of the 7 experiments the eggs in medium at pH 6.0 responded more strongly. The overall average, based on 918 eggs at pH 8.0 and 1033 eggs at pH 6.0, shows that 65 per cent formed rhizoids on the halves away from the ultraviolet at pH 8.0 while 75 per cent did so at pH 6.0. This significant difference shows that the eggs are quite appreciably more sensitive to λ 2537 Å when they are in acidified medium. The eggs used in this and certain other series of experiments were less sensitive than those used to obtain the results shown in Fig. 1, but this is not regarded

as important since changes of this order are commonly found in different collections, especially at different times.

Another group of experiments was carried out to test the effect of pH of the medium on the sensitivity of the eggs to the retarding and inhibiting effects of strong dosages. In three experiments eggs were irradiated with 30,000 units of ultraviolet. The mid-point of the period of irradiation was at 8 hours after fertilization. In each experiment eggs in two vessels were placed in normal sea water at pH 8.0, while eggs in two other vessels were placed in sea water acidified to pH 6.0 at $2\frac{1}{2}$ hours after fertilization and remained in this acidified medium thereafter. In all, 1049 eggs were irradiated and reared at pH 8.0, and 1358 eggs were irradiated and reared at pH 6.0. Non-irradiated control eggs have all formed rhizoids at 24 hours after fertilization (Whitaker, 1936), but the development of eggs irradiated with 30,000 units of λ 2537 Å is strongly retarded and inhibited. At 48 hours after fertilization, 6 per cent of the irradiated eggs at pH 8.0 had formed rhizoids, while 25 per cent of the eggs at pH 6.0 had formed rhizoids. Each experiment separately shows essentially the same relation. One of the experiments was carried further, until eggs that had not formed rhizoids began to cytolyze. In this experiment, at 130 hours after fertilization, 32 per cent of the eggs at pH 8.0 had formed rhizoids while 61 per cent of those at pH 6.0 had done so. In another experiment approximately 400 eggs were irradiated with 50,000 units at pH 8.0, and a like number at pH 6.0. At 96 hours after fertilization none of the eggs at pH 8.0 had formed rhizoids, while 7 per cent of the eggs at pH 6.0 had formed rhizoids.

These results with strong dosages of λ 2537 Å show that the eggs are less sensitive to the damaging effects of strong ultraviolet when in medium at pH 6.0 than they are in medium at pH 8.0. Acidification of the medium thus has an opposite effect on sensitivity to strong damaging doses, compared with its effect on the sensitivity to polarity determination by moderate unilateral irradiation. This opposite effect of acidification suggests that different factors are involved in the two phenomena. Absorption measurements showed that normal sea water and acidified sea water transmit λ 2537 Å equally well, and it should also be noted that non-irradiated control eggs form rhizoids, and early rhizoids grow, at essentially the same rate in medium at pH 8.0 and in medium at pH 6.0.

Discussion

The results presented in Fig. 1 show that the position of the rhizoid formation is extremely sensitive to ultraviolet light of the wave-length

2537 Å, and that the response of populations of eggs is proportional, over a wide range, to the logarithm of the dosage. This relation of response to dosage is similar to that already shown for λ 2804 Å (Whitaker, 1941), and similar to the dosage-response relation in the polarized plasmolysis of *Fucus* eggs after unilateral irradiation with λ 2804 Å (Reed and Whitaker, in press).

Auxin has been found to be present in *Fucus* eggs by du Buy and Olson (1937), and van Overbeek (1940) has shown that auxin occurring naturally in brown algae is very probably β-indole acetic acid. It was pointed out in the Introduction that destructive wave-lengths of ultraviolet might act on the *Fucus* egg primarily by inactivating or destroying auxin, and that, if so, addition of β-indole acetic acid might be expected to counteract or reduce the effect of the ultraviolet. The present experiments show, however, that β-indole acetic acid has very little effect on the sensitivity of the eggs to small doses of ultraviolet at pH 8.0, and none at all at pH 6.0. The slight effect found at pH 8.0 is not in the direction of reducing the effect of the ultraviolet. It may be due to a slight acidifying action of the β-indole acetic acid after entrance into the cell since acidity causes an increased sensitivity. β-indole acetic acid does not relieve or counteract the retarding and inhibiting effects on rhizoid formation and cell division of strong doses of ultraviolet at pH 8.0 or at pH 6.0. It thus appears that the ultraviolet does not act primarily by inactivating or destroying auxin, either in determining the developmental polarity or in inhibiting rhizoid formation.

Acidification of the medium to pH 6.0 appreciably increases the sensitivity of the eggs to the polarity-determining effects of moderate dosages of unilateral λ 2537 Å. On the other hand, acidification of the medium considerably decreases the sensitivity of the eggs to the retarding and inhibiting effects of strong dosages. This suggests that the two phenomena depend on different actions of the ultraviolet.

SUMMARY

1. When *Fucus* eggs are irradiated unilaterally with suitable moderate doses of λ 2537 Å, the rhizoids form on the non-irradiated halves of the eggs.

2. The response of populations of eggs is proportional, over a wide range, to the logarithm of the dosage.

3. When moderate doses of unilateral ultraviolet are followed by prolonged exposures to white light from the opposite sides of the eggs, the rhizoids form on the surfaces that were irradiated with ultraviolet. Therefore, the ultraviolet does not determine the position of rhizoid

formation merely by causing extensive, general damage and incapacitation of the surface upon which it falls.

4. β-indole acetic acid (auxin) has very little effect on the sensitivity of the eggs to moderate doses of ultraviolet at pH 8.0, and none at pH 6.0.

5. β-indole acetic acid does not revive eggs in which rhizoid formation and cell division have been retarded or inhibited by heavy doses of λ 2537 Å. Therefore the ultraviolet does not appear to act on the rhizoid formation primarily by affecting or destroying auxin.

6. The sensitivity of the eggs to the polarity-determining effects of moderate unilateral dosages of λ 2537 Å is considerably increased in sea water acidified from pH 8.0 to 6.0.

7. The sensitivity of the eggs to the retarding and inhibiting effects of strong dosages, on the other hand, is considerably decreased in medium acidified to pH 6.0. The two phenomena (directive effect of small doses and inhibitory effect of large doses) thus appear to depend on different actions of the ultraviolet.

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